

## Genetic Variation of Some Species of the Genus *Tagetes* sp. Using PCR-RAPD in Iraq

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### Abstract

Random Amplified Polymorphic DNA (RAPD) indicators were carried out to prepare the genetic fingerprints of six species of the genus *Tegates*. The discrepancies between the replicates of each species (their numbers and molecular sizes) were revealed after the replicates of the samples were migrated onto agarose gel and stained with ethidium bromide. 10 primers were selected, which showed different replication products among the studied species, as those primers showed 63 polymorphic bands out of 468 total bands. Each selected starter produced between 23 bands (OPD-18) to 78 bands (OPF-04). The largest number of divergent bands (15 bands) generated by the primer OPD-13, while it appeared that the least number of divergent bands (3 bands) which was generated by the primer OPD-18. The primer efficiency ranged from 0.20 in (OPD-13) to 0.09 in (primer OPA-04). It was found in this study that the primer OPD-13 had the highest discrimination ability, while the primer OPD-18 had the lowest value of the discrimination ability. The lowest genetic dimension was (0.3755) between the two species *T. erecta* and *T. patula*, while the largest genetic dimension was (0.8466) between the two species *T. patula* and *T. tenuifolia*. The phylogenetic tree through the unweighted pair-group method of arithmetic means (UPGMA) that is, based on the tree of phylogenetic relationships revealed two main genetic groups. The general analysis of the results showed that the genetic relationships between the species of the genus *Tegates* are related to some morphological traits as well as to the original origin and at the level of molecular genetics..

**Keywords:** Genetic Variation, PCR-RAPD, *Tagetes* sp

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### Introduction

According to Namtta et al (2013) The genus *Tagetes* belongs to the family Asteraceae, and this genus contains 56 species. It is native to Mexico, west to Arizona in North America and south to South America, and its cultivation spreads all over the world (Batish et al., 2007). In addition to naming the flowers of the plant with marigold, the flowers of this species are also called velvet flower, Qadifa flower, Marigold flower, or as it is called in Jaafari Iraq (Al-Sultan et al., 1992;Sadia et al., 2013).

Marigolds are among the common summer annual flowers in our gardens, and their cultivation succeeds in all parts of Iraq. The plant is characterized by its rapid growth. The flowers are beautiful in appearance and are suitable for picking. They remain on the plant for a long time, and it is less susceptible to pests and diseases due to the distinctive smell of the plant that repels insects. Its roots release substances that have a deadly effect on nematodes in the soil, and therefore it may benefit the cultivation of species of this genus in orchids that are infected with nematodes, or interchangeably with other ornamental plants that are infected with nematodes.

It is used in coordination as the short varieties of it are grown as pot plants or as determinants of flower beds, while the medium and tall varieties are grown as bedding plants (Al-Qawasmah, 2000).

Genetic variations in the plant community are the basic rule for the process of selection or hybridization within one species to produce new genetic assets. Therefore, the creation of new genetic variations will have an effective role in expanding the genetic base of the species and increasing the possibility of obtaining new varieties with distinct characteristics. The sources of variations that plant breeders work on are either natural resulting from isolates of crossbreeding between individuals of varieties and natural mutations, or artificial resulting from the use of physical or chemical mutagens (Wasswa, 2001; Shashikala, 2006).

The rapid progress in molecular biology has created many means and methods that have been used in studies and evaluation of variations and relationships between genetic structures. Indicators are abundant in numbers, quick to obtain, and unaffected by the environment, tissue type and age stage of the organism, as well as they can detect changes not only in the encoded parts of the DNA, but also in the non-coding parts, which constitute 50-90% of the genome size in higher organisms (Joshi et al., 2011).

One of the most common and recent indicators is Randomly Amplified Polymorphic DNA (RAPD), which is characterized by its use of universal primers that can be applied to the DNA of any organism. By transferring the product onto an agarose gel after staining it with ethidium bromide, the difference between the different organisms is shown in the number and size of the multiplied pieces (Duca et al., 2008).

These indicators are characterized by that they do not need prior knowledge of DNA sequences, and they do not require a long time to complete, and they are uncomplicated and do not need radioactive isotopes, which makes the possibility of their use in many countries. Thus, RAPD indicators have wide applications in diversity studies and building genetic maps (Crouch & Ortiz, 2004) and in the field of diagnosis and genetic fingerprinting (Riaz, 2006) as it had applications on various organisms, including plants (Hoisington et al., 2002; Glaszmann et al., 2010). This research aims to study genetic diversity and determine the genetic relationship between the species of the genus *Tagetes* based on the degree of genetic homogeneity between them, and to determine the genetic identity through the analysis of the genetic fingerprint for each of the species in question using the RAPD technique.

## **Methods**

### **Samples Preparation**

Six species of the genus *Tagetes* were used namely (*T. erecta* (1), *T. lucida* (2), *T. remotiflora* (3), *T. patula* (4), *T. tenuifolia* (5) and *T. Minuta* (6).

The seeds of the species were sown during the month of October 2020 in the wooden canopy of the Najaf Governorate Agriculture Department in cork dishes. After 6 weeks, the seedlings were transferred to clay pots filled with a mixture of soil. Clean leaves free from disease and insect infestations were collected for each type separately, which were used for DNA isolation and analysis using RAPD technique.

### **DNA Isolation**

Isolate DNA using a Genomic DNA Mini Kit supplied by Geneaid Biotech. Ltd; Taiwan Company, which provides an easy and quick way to obtain DNA, if you follow the instructions that come with the kit.

The amount of DNA in the samples was estimated using a device (UV Spectrophotometer, Shimadzu, Japan) in the presence of UV rays and a wavelength of 260 nanometers, and each reading of the optical density on the device was 1 equivalent to 50 micrograms of DNA/1 ml of liquid. The purity of DNA was also estimated by dividing the number of the optical density reading at a wavelength of 260 nm by the number of the reading of the optical density at the wavelength of 280 nm.

#### Preparation of RAPD reactions

Tried 20 primers from Operon technology, each consisting of ten nucleotide bases, of which 10 were selected, which were distinguished by their ability to give clear differences in DNA (Table 1).

Table 1. Primers that showed variations and their sequences

Primer	Sequence (5'→ 3')	Primer	Sequence (5'→ 3')
OPD-18	GAGAGCCAAC	OPA-02	TGCCGAGCTG
OPF-01	ACGGATCCTG	OPA-04	AATCGGGCTG
OPF-04	GGTGATCAGG	OPD-02	GGACCCAACC
OPT-17	CCAACGTCGT	OPD-7	TTGGCACGGG
OPT-20	GACCAATGCC	OPD-13	GGGGTGACGA

All random amplification reactions were carried out using Protocol of AccuPower® TLA PCR PreMix tubes containing some components of the polymerase reaction, then 5 µl of DNA sample, 2 µl of the primer were added and supplemented with sterile distilled water to 20 µl. The tubes were transferred to a thermocycler to start the replication reaction.

The replicated products are then transferred onto agarose gel (ethidium bromide-stained) at a concentration of 1.2% with a DNA ladder of 1 kilo base pair (250-10000) base pairs for 3-4 hours (70 V), and the gel is examined under ultraviolet radiation. UV-Light and imaged using an imaging device, after which the number of resulting bands and their molecular sizes are calculated using each primer (Weigand & Udupa, 1993)

#### Results and Discussion

Genomic DNA was isolated from plant leaves, and a quantity of DNA was obtained that ranged between 150-290 ng/microliter and a purity ranged between 1.8-1.9. Which indicates the efficiency of the used extraction method because isolating an amount of DNA with an appropriate purity from plants is relatively more difficult than other organisms because of the thick wall surrounding the cell membrane, in addition to the fact that some plants contain a high amount of phenolic substances and polysaccharides that may inhibit the reactions of the PCR (Pandey et al., 1996).

20 primers manufactured by Operon technology, known worldwide for the quality of their primers, have been tested to ensure accurate results. The results of using these primers in RAPD reactions have shown a difference in the number of multiplex bands and their molecular weights depending on the starter used, resulting from the difference in the number of sites The complement to that primer in the genome of each type of amaranth plant used in this study, and this agrees with all the research published in this field, including for example (Williams et al., 1990).

Since the aim of the study is to reveal the genetic variations between the species used in this study, ten primers that give common bands were removed and only ten of the primers were adopted in the analysis of the products of RAPD reactions (Table 1), meaning that the number

of primers that show different replication products in RAPD interactions depend on the type of template DNA (Vanova, 2000).

The prefixes differed in their ability to generate the number of multiple pieces (bands), as it was found that the highest number of bands was obtained using the primer OPF-04 and the number of bands resulting from its use was 78, followed by 73 bands obtained from the use of the primer OPD.13, 66 bands were obtained It was obtained from using the OPD.02 primer and then 51 bands obtained from using the OPA.02 primer (Fig. 1), and by analyzing the results it was found that the highest number of dissimilar bands for the OPF-04 primer is 15 and the primer OPD.13 is 9 (Table 2).

The lowest number of duplicated bands was 28 and 23 bands appeared using the OPD.07 and OPD.18 starters respectively, and the lowest number of differentiated bands for the OPD.18 primer is 3 bands.

It is important to know that some primers have the ability to recognize a large number of annealing sites (correlation) which is more useful than primers that recognize a smaller number of bonding sites, in this case the number of multiplexing beams will be higher, thus giving a better chance of detection On the variation in DNA between individuals (Williams et al., 1990).

The presence of the complementary site of a particular sequence in a particular type of DNA without the rest of the species is due to the fact that there are certain sequences that are repeated in a type of living organism and other sequences that are rare and non-existent (Burge et al., 1992).

The primer efficiency ranged from 0.20 in the OPD-13 primer to 0.09 in the OPA-04 primer. It was found in this study that the primer OPD-13 had the highest value in the ability to discriminate, which is 23.80, while the primer OPD-18 showed the lowest value, which is 4.76 (Table 2).

The results of discriminative power can be expressed through the ability of primers to reverse variation in an individual's genome according to the total number of heterozygotes. Therefore, I noticed that the primer that has a high ability to bind also has the ability to give the largest number of heterozygous bands.

Several reports have pointed out the importance of determining the discriminatory competence and power (high ability to relate) in any primer to calculate the genetic variance of the species under test (Khampila et al., 2008a).

The lowest genetic dimension was (0.3755) between the two species *T. erecta* and *T. patula*, while the largest genetic dimension was (0.8466) between the two species *T. patula* and *T. tenuifolia* (Table 3).

The synthesis analysis revealed (phylogenetic tree) through the unweighted pair-group method of arithmetic means (UPGMA), that is, based on the tree of evolutionary relationships, into two main genetic groups, the first group included *T. lucida* and *T. patula*, while the second group included four species: *T. erecta*, *T. remotiflora*, *T. tenuifolia* and *T. Minuta* (Fig. 2).

And that the presence of a percentage of high genetic similarity between species is at most due to their sharing of the same alleles that descended to them from a common ancestor, and on this basis the genetic relationship was adopted (Esselman et al., 2000;Priyanka et al., 2013). When reviewing the phenotypic characteristics of the two species, it was found that they They share many phenotypic traits, such as plant height and flower color, and from this it is concluded that the presence of common phenotypic traits contributes to increasing the

percentage of genetic similarity between the samples studied using this type of indicators and is consistent with what was found by Bai (Bai & Reeleder, 1997).

It is also in agreement with Degani et al (2001) which found that the degree of genetic similarity that depends on the indicators of RAPD depends a lot on the geographical location and the origin of the species under study.

The existence of genetic similarity between certain species present in the same site based on RAPD indicators has a logical explanation as they are genetically adapted to the environmental conditions prevailing in that site (Del-Rio & Bamberg, 2000) so the genetic material in it is somewhat similar, which is reflected in the number of The common packages between them and this was also reached by Hormaza (Hormaza et al., 1994) with a high degree of similarity between pistachio cultivars found in a particular site compared to other sites based on RAPD indicators.

The importance of finding the genetic dimension of the plant breeder who wants to benefit from genetic analyzes between species at the level of DNA, for example, is chosen as the two genetically farthest species from each other as parents to conduct breeding operations to allow obtaining the largest possible number of widest possible crosses. In other cases, the breeder may wish the introduction of a specific trait that is controlled by a gene or a group of genes for a particular variety without a significant change in the genetic material for that variety that contains desirable traits phenotypic (Smith et al., 1992;Mor et al., 2008).

Finding the genetic relationship between the species of the genus *Tagetes* present in Iraq for the first time opened broad prospects for conducting other studies at the DNA level for these plant species, especially in the field of breeding based on genetic studies, especially if it is known that the same data can be used for the purposes of screening species to search for Gene encodes as desirable for example.

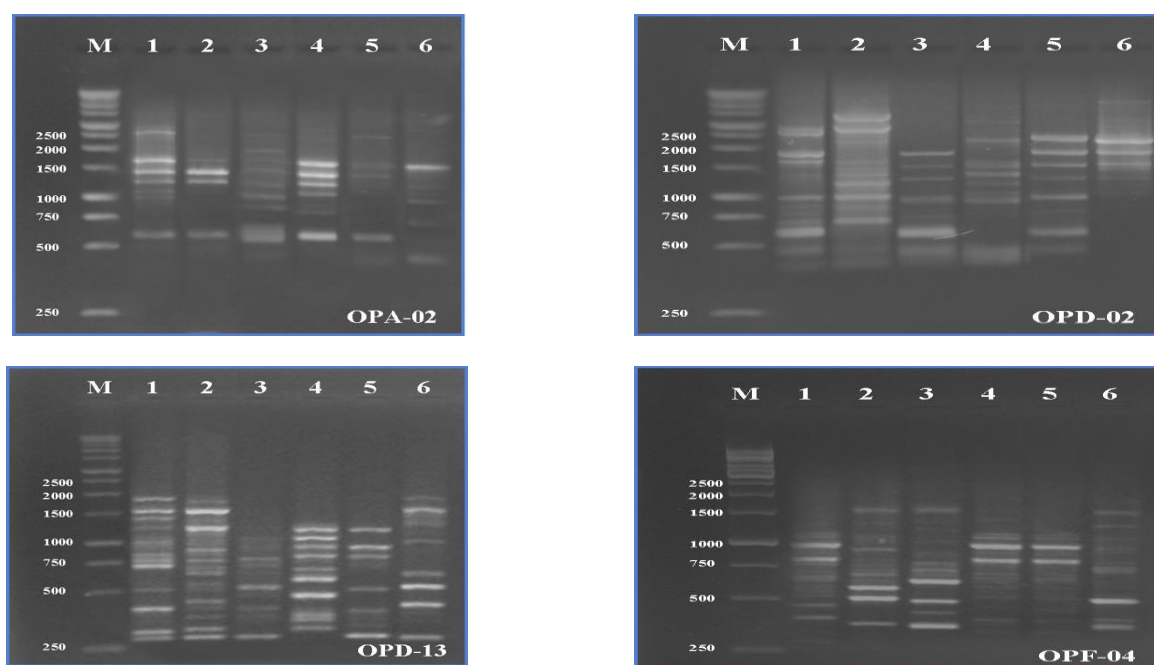


Figure 1. Replication products (OPA-02, OPD-02, OPD-13 and OPF-04) staged on agarose gel at a concentration of 1.2% with a DNA ladder (M) and numbers 1-6 representing the species of the genus *Tegates*.

Table 2. It represents the type of primer, number of total bands, and number of variation bands, primer efficiency and discriminant value.

Primer	Number of total bands	Number of variation bands	Primer efficiency	Discriminant value
OPA-02	51	5	0.10	7.93
OPA-04	43	4	0.09	6.34
OPD-02	66	7	0.10	11.11
OPD-7	28	4	0.14	6.34
OPD-13	73	15	0.20	23.80
OPD-18	23	3	0.13	4.76
OPF-01	31	5	0.16	7.93
OPF-04	78	9	0.11	14.28
OPT-17	40	5	0.12	7.93
OPT-20	35	6	0.17	9.52
	468	63		

Table 3. Genetic distance among the genus Tegates studied using RAPD analysis.

Species	1	2	3	4	5	6
1	0.0000					
2	0.7013	0.0000				
3	0.7442	0.5510	0.0000			
4	0.3755	0.8253	0.5970	0.0000		
5	0.4180	0.6025	0.6021	0.8466	0.0000	
6	0.5623	0.5647	0.6112	0.7180	0.7223	0.0000

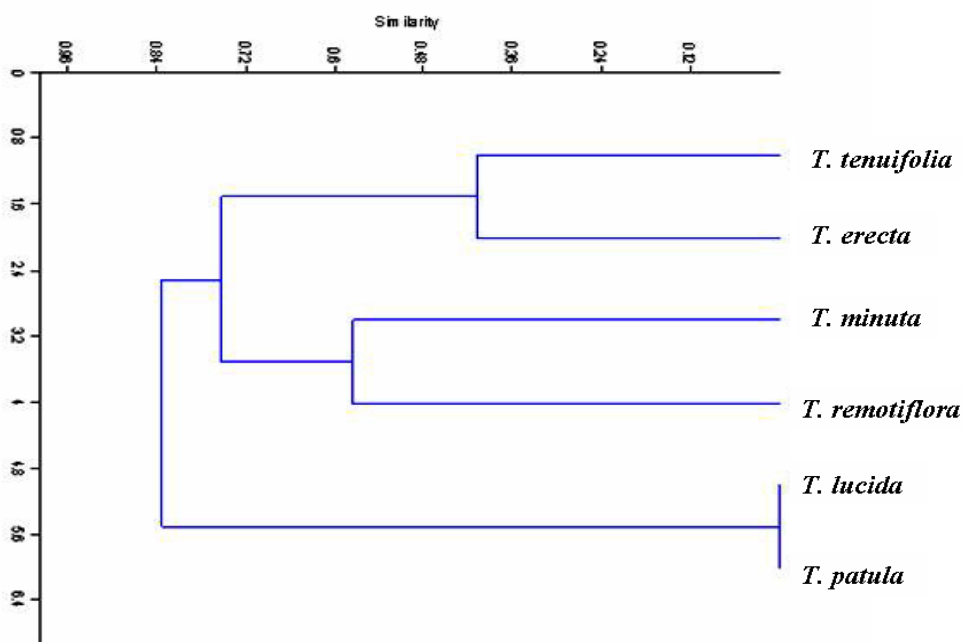


Figure 2. The Dendrogram represents the genus Tegates was carried out using RAPD analysis

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